Sandia National Laboratories' portable µChemLab™ Biological Agent Detection Sensor

Sandia's µChemLab™ Bio Detection System is a transition-ready technology that directly addresses HSARPA requirements for BAA 05-06, Food Biological Agent Detection Sensor (FBADS), including:

- Low limits of detection (1 pM; equivalent to 0.05 nanograms Ricin/ml) using laser-induced fluorescence detection with automated sample preparation
- False positives and false negatives reduced using internal standards and sophisticated signature analysis software
- Automated sample processing (one step) for ease of use by non-technical personnel
- Low cost of ownership – no expensive reagents
- Modular design enabling straightforward component replacement and upgrades
- Ability to simultaneously detect multiple agents and toxic products
- Rapid analysis, typically in 5-10 minutes
- Ability to identify agents attached to particles

µChemLab™ is a lab-tested prototype, ready to transition to industry, with additional development and testing needed to make it ready to transfer to the field.

BAA 05-06 ultimately seeks to detect NINE agents; µChemLab™ has already been demonstrated in a laboratory configuration to detect and identify a number of bioagents including FIVE of these (highlighted in red below):

- ☑ Clostridium botulinum neurotoxin (surrogates)
- ☑ Staphylococcal enterotoxins (A and B)
- ☑ Bacillus anthracis
- ◆ Francisella tularensis
- ☑ Yersinia pestis
- ☑ Escherichia coli
- ◆ Shigella dysenteria
- ◆ Salmonella spp
- ◆ Brucella abortus
- ◆ The instrument has also demonstrated the ability to detect Cholera toxin subunits and Tetanus toxin.

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Transition-ready technologies at Sandia National Laboratories for HSARPA BAA 05-06 Food Biological Agent Detection System (FBADS)

Partnering opportunities with Sandia National Laboratories

With long-term programs in chemical and biological national security, Sandia National Laboratories has developed a hand-portable micro-analytical instrument based on "lab-on-a-chip" technology that could accelerate and enhance industry solutions to the challenges posed in the HSARPA Food Biological Agent Detector Systems (FBADS) Broad Agency Announcement 05-06 (BAA 05-06).

Collaborating with Sandia to integrate these technologies into national security solutions offers industry—and the nation as a whole—a number of benefits:

- **Faster, more economic development cycle**: Through decades of exploring national security issues, Sandia scientists have built up considerable expertise. Organizations that can tap this expertise will develop solutions more quickly and at less cost, ultimately accelerating the nation's ability to respond to national security challenges.
- **More robust solutions:** Sandia applies its renowned expertise in systems analysis and engineering to create practical, enduring solutions for the real world. By insisting that development efforts take into account the often less-than-ideal deployment context, Sandia can help industry develop more robust solutions.
- **Innovation through interdisciplinary teams:** Sandia scientists and engineers routinely work across disciplines to solve difficult challenges. Industry partners with reach into this profound blend of expertise can discover new opportunities for innovative solutions.
- **Long-term solutions**: As a national laboratory, Sandia is charged to perform the long-term research that is beyond the reach of most industrial entities. A partnership with Sandia on one project could lead to additional collaborations to create long-term solutions to today's problems and tomorrow's challenges.
- **Leveraged ROI:** Sandia technology development results from multi-year investments by federal agencies. Use of these technologies in solutions that bolster national security provides industry—and the taxpayer—an excellent return on investment.

This document will describe in detail Sandia's µChemLab™ and supporting capabilities that are relevant to this BAA. It then provides a brief overview of Sandia National Laboratories' comprehensive Chemical and Biological National Security program. The final section summarizes HSARPA guidelines and restrictions governing bidder partnerships with Sandia National Laboratories.

(For more information on Sandia's Chemical and Biological National Security program, please visit our website: www.ca.sandia.gov/chembio)

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µChemLab™ Bio Detection System

Several key technologies are related to the µChemLab™ bio detection system. Sandia National Laboratories is developing fully self-contained, portable, hand-held chemical analysis systems incorporating *"lab on a chip"* technologies. Our µChemLab systems utilize microfabricated substrates to provide sensitive devices with fast response times in a low power, compact package. Currently, devices are being developed and tested for the detection of chemical and biological warfare agents, with the potential for analyzing chemical and biological compounds for multiple defense, environmental and medical applications.

Sandia researchers have miniaturized laboratory chemical analysis. The hand-held µChemLab™ Bio Detector spots molecules as dilute as 1 in 10 billion in less than 10 minutes and runs 8 hours on 4 watts of power supplied by lithium camera batteries. When combined with automated "front-end" preconcentration components we have developed, detection limits as low as 1 pM (equivalent to 0.05 ng ricin/ml) can be achieved. Multiple, microfabricated channels in the device separate molecules on the basis of charge/mass ratio and size using small-scale versions of a standard analytical approach, electrophoresis. A laser diode excites fluorescence in the dye-labeled molecules as they emerge. On-board data processing identifies analytes of interest.

Key transition-ready technologies and supporting capabilities related to µChemLab™ include:

- The entire integrated system
- Underlying modules, including sample preparation systems, fittings, valves, pumps, mixers, concentrators, lyser/solubilizers, and optical subsystems
- Rapid "plug and play" design, assembly, and testing of novel concepts based on component architectures
- Design and engineering to produce prototypes of systems and underlying modules

The sections below offer greater detail on µChemLab™ and its components.

µChemLab™ System Design and Operation

Sandia has developed a hand-portable microchip-based analytical instrument, referred to as µChemLab™, for the detection and identification of proteins and other biomolecules.

Proteins are labeled with a fluorescent dye (typically fluorescamine) and separated on a microfluidic chip from other solution components according to molecular weight, using capillary gel electrophoresis (CGE), and mass/charge ratio using capillary zone electrophoresis (CZE). Following the separation, which typically occurs in 3 to 7 minutes, the labeled proteins are detected using laser-induced fluorescence (LIF), which provides fundamental (without preconcentration) picomolar range (10^{-11} M) detection sensitivity of fluorescent dyes and nanomolar sensitivity (10^{-9} M) for fluorescamine labeled proteins.

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Proven Capabilities

To date, we have demonstrated the detection and identification of biotoxins such as ricin, SEA, SEB, and tetanus; T-even phage bacterial viruses, as well as MS2, alpha encephalitis, and Vesicular Stomatitis, and Vaccinia viruses; and B. anthracis spores and vegetative cells.

Figure 1 shows the µChemLab™ hand-held modular bioagent detection device

Design

As shown in Figure 1, the µChemLab™ device has a modular design that provides reliability and flexibility, and facilitates rapid assembly, fluid and microchip replacement, troubleshooting, and sample analysis. Components include two independent separation modules that incorporate interchangeable fluid cartridges, a 2-cm-square fused-silica microfluidic chip, and a miniature LIF detection module. A custom o-ring-sealed manifold plate connects the chip access ports to a fluids cartridge and a syringe injection port, providing sample introduction and world-to-chip interface. Other novel microfluidic connectors include capillary needle fittings for fluidic connection between septum-sealed fluid reservoirs and the manifold housing the chip, enabling rapid chip priming and fluids replacement. Programmable high-voltage power supplies provide bidirectional currents up to 100 µÁ at 5000 volts, enabling real-time current and voltage monitoring, and facilitating troubleshooting and methods development.

Analysis Methods

To enable rapid identification of bioagents with low false positive/negative rates, we have applied principal-component analysis techniques that can, for example, distinguish between spores and vegetative cells. These methods are currently being augmented by algorithms based on Bayesian analysis methods, which can extract signatures from noisy data and thus improve the ability to separate signatures of interest in the presence of interferents. In addition, we developed a novel two-color labeling system, in which red-labeled standards are simultaneously detected in the presence of blue-labeled protein analytes. This allows inevitable variations in protein migration times to be corrected, dramatically reducing the potential for false positives/negatives.

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µChemLab™ subsystems

In addition to the analytical capabilities of µChemLab, we developed front-end components that allow automated sample processing including dye labeling, buffer adjustment, lysing, and preconcentration. The following component prototypes have been tested:

- Microfluidic solid-phase extraction cartridges for protein preconcentration
- Thermal- and chemical-based units capable of lysing viruses and spores
- Automated in-capillary protein labeler
- Size exclusion cartridge for separating proteins from small molecules.

An integrated prototype (breadboard) system including these components as well as pumps, automated valves, and computer control has been demonstrated using biotoxins. Complete systems for processing spores, and vegetative cells are under development. Liquid samples containing pathogens are automatically injected into the system, solubilized, labeled, and separated. The pathogens are then identified from their characteristic protein signatures.

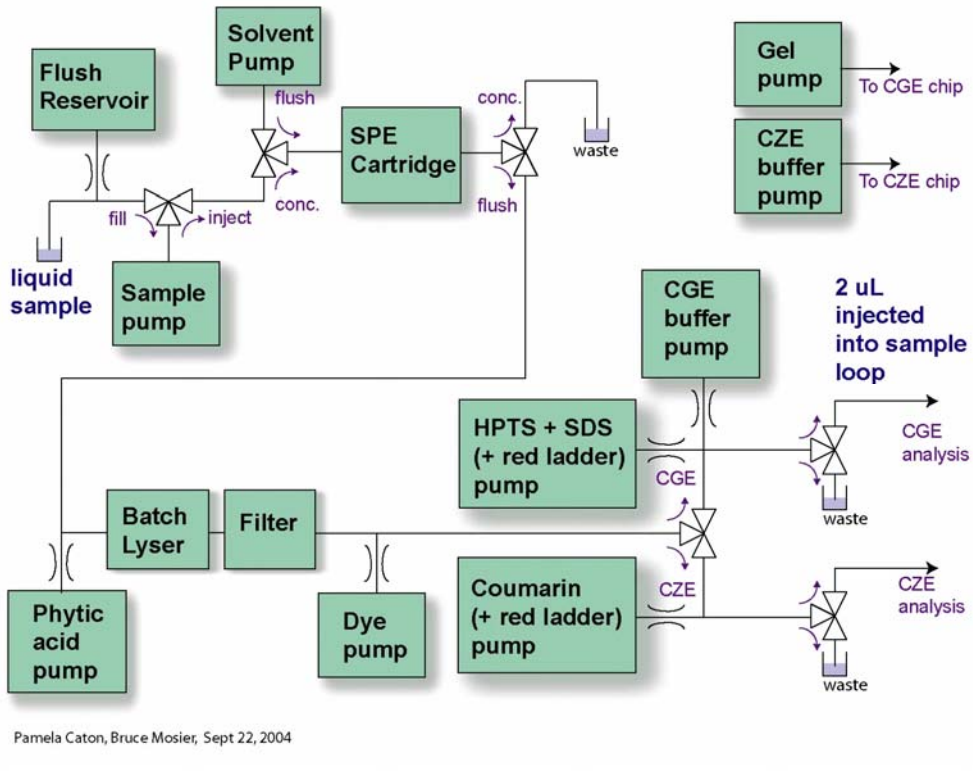

Figure 2 shows a schematic of the key elements of our µChemLab™ approach

Figure 2 shows a schematic of a prototypical sample processing system. We expect that we will be able to sort classes of pathogens using iDEP (see below). Each pathogen will then be processed through a system analogous to the one shown in Figure 2. Spores, vegetative cells, viruses and biotoxins are expected to be processed simultaneously but separately. During the

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past year we have demonstrated use of these microfluidic sample preparation components individually and in some cases on a system level in an integrated breadboard as shown in Figure 3.

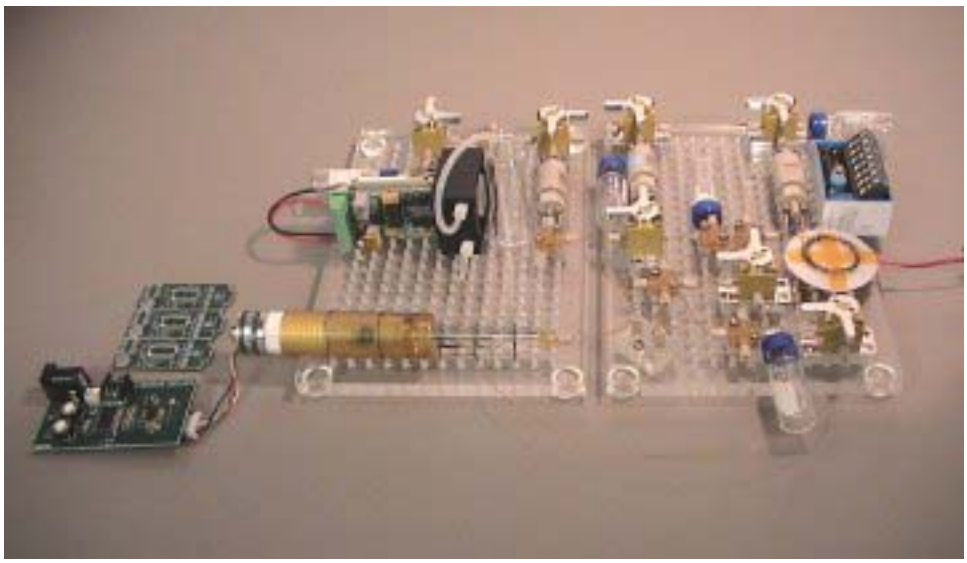

Figure 3 shows a breadboard sample preparation system, which includes all of the components needed for this approach as well as some that are no longer being considered.

Lysis – For this application, lysis involves more that just opening the cell; the constituent proteins must be solubilized. For vegetative bacteria and viruses, there are several options for solubilization. Spores, however, are much more difficult to solubilize. Spore coat proteins form a protective and highly cross-linked surface structure. Physical disruption methods such as heat or pressure can break open the structure so that DNA can be extracted, but physical disruption alone is not sufficient for recovering proteins. A chemical reducing agent is needed to break apart the disulfide linkages. In our subsystem, a detergent is then used to solubilize the reduced proteins. In the past year, we have demonstrated effective lysis and solubilization of bacillus spores using either ß-mercaptoethanol (BME) or Tris[2-carboxyethyl] phosphine (TCEP) as reducing agents. The unit can operate at temperatures near boiling to speed the process. The lysis/solubilzation process has been demonstrated as both a bench-top procedure and in a micro-fluidic device. The device includes a temperature and pressure controlled lysis chamber of approximately 10 µL volume and size exclusion chromatography (SEC) cartridges for subsequent cleanup of the reducing agent.

Size Exclusion Chromatography – Prior to injection into the µChemLab, the proteins are labeled with fluorescamine. The highly charged TCEP required for lysis of spores interferes with the subsequent labeling step. For this reason, we have developed miniature size exclusion chromatography (SEC) cartridges which remove TCEP in a flow-through mode. In this application, SEC is not used to separate proteins for detection or identification; its only function is to remove TCEP in preparation for the subsequent labeling step. In SEC, the components of a mixture are separated according to their molecular size, based on flow through a porous packing. Large molecules (proteins in this case) are excluded from interacting with the pores of the

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packing material, and therefore flow through the cartridge quickly. Small molecules (TCEP) enter the pores and flow through the cartridge more slowly since their path through the column is longer.

Labeling – Labeling with fluorescamine dye is accomplished by simultaneously flowing the proteins and the dye stock solution (10 mM in dry acetonitrile) through a capillary tube in proportions such that the final fluorescamine concentration is 1 mM. This capillary-based method is comparable to the benchtop labeling method.

Miscellaneous components – In addition to the specific components described previously, the sample preparation system has pumps, valves, power supplies, and controllers, all of which are shown in Figure 3. All of these microfluidic components have been developed and demonstrated.

Dielectrophoresis – Sandia's range of dielectrophoretic filter/concentrators are demonstrated to concentrate by >6000X; sort different species of live bacteria and spores (Figure 4); and separate living bacteria from dead bacteria (Figure 5) in a laboratory setting. The system has not yet been used to deliver sorted/concentrated specimens to subsequent sample handling subsystems like the ones depicted in Figures 2 and 3, The novel approach employs microfabricated or replicated insulating obstacles to produce non-uniformities in an electric field that is applied via remote electrodes. Called "insulator-based" dielectrophoresis (iDEP), this methodology will allow devices to be constructed from injection-molded or stamped plastics without further microfabrication steps. The devices can be operated at electric field frequencies down to D.C., allowing electrokinetic driving and selectivity based on size, surface charge density, and the electrical properties of the cell membrane, internal membranes, and other cellular material.

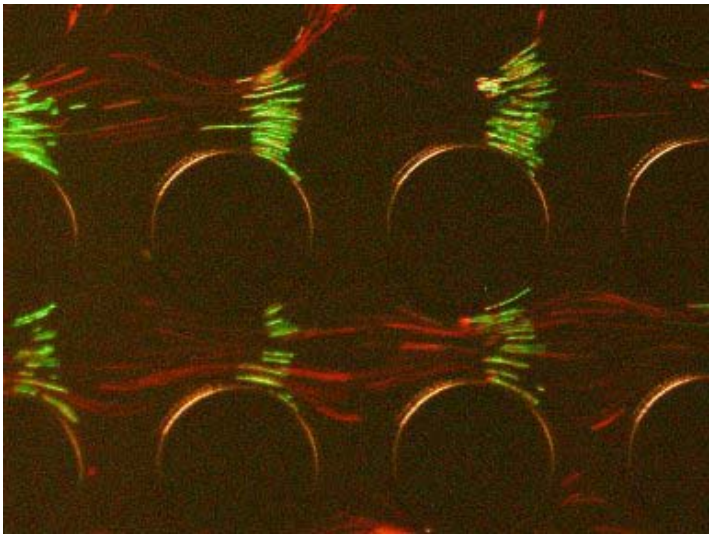

Figure 4 Selective dielectrophoretic trapping of Bacillus cereus (green) vs. Bacillus subtilis (red). Flow is from right to left at ~1 mm/s. The glass insulating circular posts are on 200-µm centers.

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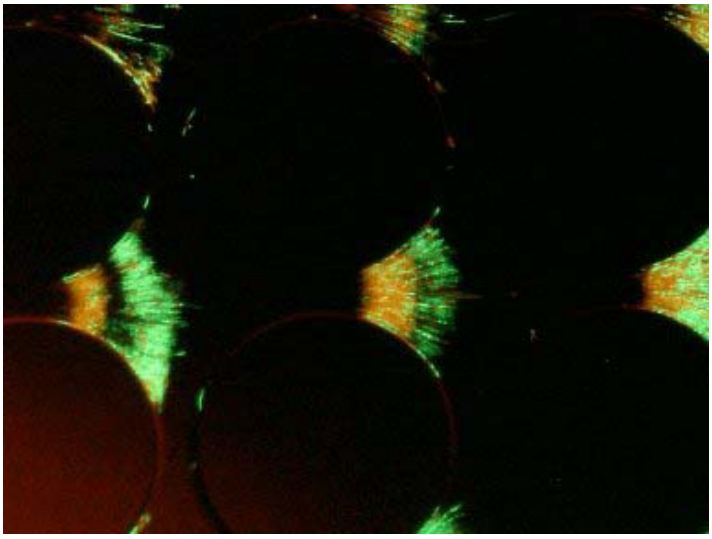

Figure 5 Dielectrophoretic sorting of live (green) and dead (orange) E. coli. Flow is from right to left at ~1 mm/s. The glass insulating posts are on 200-µm centers.

(For more information on Sandia's µChemLab™ systems please go to: www.ca.sandia.gov/chembio/tech_projects/detection/micro-chem-lab.html)

Sandia National Laboratories Chemical and Biological National Security Program

Sandia National Laboratories is a multi-program engineering and science laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the U.S. Department of Energy (DOE). Sandia also works for the Department of Homeland Security (DHS), Department of Defense (DoD), and other federal agencies and partners with other federal groups, universities, and private industry. Our national security mission has grown from responding to the threats of the Cold War to countering a host of new threats—some nuclear, others involving chemical, biological or radiological weapons of mass destruction, and still others that are acts of terrorism.

Building on its systems integration strengths, Sandia National Laboratories has developed a comprehensive Chemical and Biological National Security Program. Coupling innovative science with sound engineering, technology, and systems analysis expertise, Sandia develops, demonstrates, and delivers technologies to detect, deter, defeat, or mitigate the impact of chemical or biological attacks. The broad program, underway since 1996, employs our unique capabilities to analyze, create, and integrate defensive systems. Capabilities in chemical and biological defense span basic investigations in biology and detection; expertise in systems analysis, engineering and computing; and training facilities for public health and emergency responders. Sandians have applied their expertise to projects addressing sensing, restoration, facility hardening, training scenarios, and enhanced exchange of public health data.

(For more information on Sandia's Chem/Bio Program please go to: www.ca.sandia.gov/chembio)

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Partnering Guidelines

The Sandia capabilities/technologies listed in this document are relevant to HSARPA Instantaneous Bio-Aerosol Detector Systems (IBADS) Broad Agency Announcement 04-18 (BAA 04-18) and are available to any bidder who is interested in partnering with Sandia National Laboratories under the following HSARPA guidelines/restrictions:

- DHS strategic partner laboratories may not propose directly to this solicitation or participate in any manner in the development of responses to this solicitation outside of the process defined by HSARPA.
- DHS strategic partner laboratories may collaborate with HSARPA bidders by providing explicitly identified transition-ready technologies subject to DoE and DHS approval. It is on the initiative of the providing laboratory to identify which technologies are transition-ready.
- DHS strategic partner laboratories may collaborate with HSARPA bidders by providing explicitly identified and unique supporting capabilities subject to DOE and DHS approval. It is on the initiative of the providing laboratory to identify which supporting capabilities are available to HSARPA bidders.
- HSARPA will neither encourage nor discourage bidders from incorporating DHS strategic partner laboratory technologies. This inclusion of these technologies is at the sole discretion of bidders in their evaluation of best value and best technical response to the government under this solicitation.

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